PROJECT NUMBER: 1730
PROJECT TITLE: Plant, Cell & Tissue Culture Research
PROJECT LEADER: I. L. Uydess
PERIOD COVERED: July, 1988

I. TOBACCO-IDENTICAL PRESERVATIVES:

A. **Objective**: To develop procedures and to establish microbiological screens for the evaluation of new, nature-identical preservatives as replacements for and/or as adjuncts to propylparaben.

B. **Status**:

1. **Phase I Preservative Screens**:

a. A 1:1:1 mixture of C-10, C-11 and C-12 fatty acids (to a final concentration of 100 µg/ml) has been tested in the Phase I shake flask assay using B. coagulans as the target organism and 100 µg/ml each of decanoic acid and propylparaben as the controls. The fatty acid mixture was observed to have approximately the same antimicrobial (bacteriostatic) effect as the 100 µg/ml decanoic acid control, producing complete inhibition of growth over the 24 hour time course of the experiment. The 100 µg/ml paraben control, on the other hand, yielded only a marginal inhibition of growth compared to the preservative-free control.

b. A set of shake-flask experiments was conducted to further evaluate the antimicrobial nature of decanoic acid. Lag, mid-log and late stationary phase cultures of B. coagulans were exposed to 100 µg/ml decanoic acid (in PG) in an attempt to differentiate the bactericidal vs bacteriostatic impact of this C-10 acid. Representative samples of each culture as well as a preservative-free control were plated prior to and 40 minutes after the addition of the decanoic acid to obtain viable cell (CFU) counts). The OD_{650} of each flask was also followed over this same interval. Metabolically-active (lag) and rapidly-growing (log) phase cells were found to suffer a 0.5 - 1.5 log loss in CFUs after the addition of decanoic acid while the CFUs of non-growing, (late-) stationary cultures remained the same. This loss in viability was paralleled by decreasing OD_{650} readings for the lag and log-phase cultures over this same interval. Thus, while the fatty acids are believed to act primarily as bacteriostatic agents (1), it would appear that decanoic acid can be cidal to a substantial portion of these bacterial populations depending upon the state and conditions of growth.

c. Spores of several ATCC strain types and PM isolate cultures were exposed to 100 µg/ml decanoic acid in a Phase I screen in order to determine if this C-10 fatty acid was inhibitory to germination. In repeated trials, decanoic acid was observed to inhibit the outgrowth/proliferation of cells

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from all of the spore types tested over a 48 hour period. Subsequent to 48 hours, however, two of the seven spore types examined (B. coagulans and PM Isolate #10A) were observed to germinate based upon an increase in the OD_{650}. Germination was not observed in the remaining strains (including B. brevis, B. licheniformis, B. subtilis, and two unidentified SEL isolates) up to and including 96 hours of incubation.

2. **Microbiological (Phase II Screen) Investigations**:

A series of preliminary, Phase II preservative screens were initiated during this period using C-Pilot Plant SEL. The fresh SEL was first plated for initial spore and vegetative cell (CFU) counts and then dosed with various levels of decanoic acid up to and including 300 µg/ml. All flasks (+/- preservative) were then incubated at 37°C either standing or shaking @ 150 rpm in order to induce spoilage. Propylparaben in PG served as a control. CFU and pH determinations were made at 24 hour intervals over the course of the experiment. Initial observations indicated that decanoic acid @ 250 - 300 µg/ml inhibited the progression of spoilage in the C-Pilot Plant SEL under these conditions [the pH of the + decanoic acid flasks remained unchanged over the duration of the experiment compared to the rising pH of the non-shaking ("spoiling") controls]. Furthermore, several of the colony types observed in the preservative-free and paraben control flasks were found to be absent in the flasks containing 250-300 µg/ml decanoic acid.

C. **Conclusions**: None to be reported at this time.

D. **Plans**: **August, 1988**

1. To continue the lab-based evaluation of the C-10 thru C-12 fatty acids in Phase II screens using Pilot Plant and Park 500 SELs.

2. To initiate fermentor-based (Phase III) screens of the targeted fatty acids.

E. **Reference**

1. Freese, E., Sheu, C. E. and E. Galliers, "Function of Lipophilic Acids as Antimicrobial Food Additives", Nature, 241:321-325, 1973.

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